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COMPARATIVE VALUE, FROM STANDPOINT OF
PUBLIC HEALTH, OF SMEARS, CULTURES AND
COMPLEMENT FIXATION IN THE DIAG-
NOSIS OF CHRONIC GONORRHEA
IN WOMEN *

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In the first part of this article are reported the results which have been obtained in the application of the cultural methods of Torrey and Buckell¹ to the diagnosis of cases of chronic gonorrhea in women, and in the second part, a comparative study of the relative values, from public health standpoint, of smears, cultures and complement-fixation tests in the diagnosis and control of such cases is presented. The patients were prostitutes who had received a court sentence.* *

1. *Cultural Results.*—As it was not feasible to seed the culture plates immediately after obtaining specimens from these cases, it was necessary to devise a procedure through which the viability of any gonococci present in the discharges, on the swabs, would be preserved during the period occupied in transporting them to the laboratory. A method which proved satisfactory is described in the article referred to. The plates should be poured at least 2 or 3 hours before they are to be streaked. After seeding, they are placed in an incubator in which the air is kept very moist and the temperature at 36 to 37 C. It is seldom worth while to attempt fishings from the plates after only 24 hours' incubation as the gonococcus colonies are frequently very small—barely visible to the naked eye—and not particularly characteristic. After 48 hours, however, the colonies have generally assumed a typical appear-

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** The material for culturing was obtained either at the clinic connected with the Court or at the Kingston Avenue Hospital for Communicable Diseases in Brooklyn. The complement fixation tests were made by Miss M. A. Wilson, in charge of the Health Department Serologic Laboratory, and smear diagnoses by Dr. Catherine Regan, Bacteriologist at the Kingston Avenue Hospital. In correlating the work we have received assistance from Dr. W. H. Park and Dr. Anna Williams.

¹ Jour. Infect. Dis., 1922, 31, p. 125.

ance and those resembling the gonococcus type should be fished. It is best not to delay the fishing from medium B plates beyond the second day as the gonococci tend to die out rather quickly on this medium. In some instances the gonococcus colonies have grown up very slowly, and accordingly all the plates should be incubated and inspected daily for at least 5 days. The isolation of the gonococcus in pure culture in some cases was a matter of considerable difficulty owing to the presence of large numbers of other colonies on the plates. Pure strains were always obtained, however, by replatings on one or the other of the dye-containing mediums. The appearance of the gonococcus colonies on these mediums, the method of fishing the colonies and the criteria for the identification of the gonococcus have been discussed in the preceding paper.

For each case 4 plates were generally used, prepared as follows:

1. Medium B, 10 c c plus 5 c c ascitic fluid plus 1 c c methyl violet, 1:100,000 dilution in distilled water.
2. Medium B, 10 c c plus 5 c c ascitic fluid.
3. Medium A, 10 c c plus 5 c c ascitic fluid plus 0.5 c c iodine-green, 1:3,000 dilution in distilled water.
4. Medium A, 10 c c plus ascitic fluid, 5 c c.

As is indicated in table 1, rather more successful isolations were effected with plates 3 than with any of the other combinations, although plates 2 yielded nearly as good results and in 4 instances showed gonococcus colonies when the other plates were negative. These 2 plates, however, were more frequently overgrown with contaminating bacteria.² In only 2 instances was a successful isolation obtained from plate 1 when the other 3 proved negative, and in many instances no gonococcus colonies developed on plate 1 when one or more of the other plates were positive. Streptococci gave a good deal of trouble and were present in much greater numbers in the specimens from the cervix uteri than in those from vulvovaginitis in children or from cases of urethritis in males. The methyl violet, in the strength employed, frequently did not inhibit the development of the streptococci nor certain prevalent types of diphtheroids, but too often did inhibit the growth of gonococci. It would seem, perhaps, hardly worth while to employ plates 1 in routine work on women. Plates 4, also, proved less effective than did the same medium with the dye, iodine-green. In view of these

² An effective method for limiting the overgrowth of plates with spreading types of bacteria (*B. subtilis*, etc.) consisted in ringing such colonies with gentian violet stain, applying the stain with a swab around the colony.

results we would recommend the use of mediums 2 and 3 in connection with gonorrheal cases of this type, employing at least one plate of the former and two plates of the latter for each specimen.

Of the total 102 women from whom cultures were made, successful isolations were effected in 29, or 28.4%.

TABLE 1
RESULTS OBTAINED WITH THE FOUR VARIETIES OF MEDIUMS EMPLOYED IN THE ISOLATION OF GONOCOCCUS

Case	Plate 1 Asceitic Agar B + Methyl Violet	Plate 2 Asceitic Agar B	Plate 3 Asceitic Agar A + Iodin-Green	Plate 4 Asceitic Agar A
L. Sm.	—	+	—	—
L. S.	—	+	—	—
P. A.	—	—	—	+
E. B.	—	—	+	—
N. C.	—	—	+	+
M. D.	—	+	+	—
L. R.	—	+	+	+
L. D. cerv.	+	+	+	—
ureth.	+	+	—	—
S. B. cerv.	—	—	+	—
ureth.	+	—	—	—
A. R.	—	C	+	C
A. M.	—	+	+	+
G. S.	—	—	+	+
A. Mac.	+	+	+	+
F. H.	+	+	+	+
C. J.	—	—	+	—
J. F.	—	C	+	—
M. Do.	+	+	+	+
H. M.	—	+	+	—
R. G.	—	C	+	C
R. C.	—	+	—	—
A. D.	—	+	+	—
M. H.	—	+	+	—
M. W.	+	+	+	—
J. V.	+
V. M.	—	C	+	C
R. Ca.	C	+	C+	—
G. W.	—	+	—	—
V. G.	—	+	+	—
L. S.	—	—	+	—

+ indicates gonococcus colonies on the plate; C indicates plate badly contaminated.

The strains isolated from our 29 positive cases were definitely identified as gonococci. In 5 instances pure cultures were obtained and identified on the basis of type of colony growth, morphology, and reaction to gram stain, rapid autolysis and short period of viability, but the strains were lost before further tests could be applied. The other 24 strains were all subjected to fermentation tests and a considerable number of them to agglutination tests with a polyvalent gonococcic serum. We believe there can be no question in regard to the identity of them all.

From the standpoint of control of venereal infection it is of interest to find that 9 of the 29 patients with positive cases harbored the gonococcus in the cervix uteri without any definite symptoms of gonorrhea. Also, although in most of these positive cases the number of viable gonococci was very small, there was no definite correlation between the number of gonococcus colonies on the plates and the clinical picture; that is to say, a few of the clinically doubtful cases gave fairly numerous gonococcus colonies on the plates, whereas certain other cases showing quite definite clinical symptoms yielded exceedingly few gonococcus colonies and those only on one plate. We have made no comparative virulence tests on the gonococcus strains isolated. It is possible that those from the clinically doubtful cases would prove to be relatively avirulent. Jötten³ has recently reported that strains from cases of gonorrhea with complications are, as a rule, much more virulent, as indicated by the inoculation of white mice, than those cases with milder symptoms.

In regard to 92 of the patients there are official records of the results of gram-stained smears. Accordingly, we may present at this point a comparative statement bearing on the relative values of these two methods of diagnosis. In most instances smears from the urethra and the cervix uteri were prepared at the time the specimen for culture was obtained. In addition, from most of the cases smears were taken at other times. In the tabulation the report most suggestive of gonorrhoeal infection has been selected. In diagnosing these smears the rules of Williams and Wilson⁴ were followed: positive smears show leukocytes filled with morphologically typical gonococci; suspicious smears show some intracellular organisms suspiciously like gonococci and 50% or more of polymorphonuclear leukocytes; observation smears show 50% or more of polymorphonuclear leukocytes, but no suspicious intracellular diplococci.

Of these 92 different cases, 26, or approximately 28%, yielded positive cultures. Among these cases there was one acute infection and 8 subacute. The majority, or 59, presented the clinical picture of chronic gonorrhea, as described by Smith and Wilson,⁵ with at least 21 cases for which a diagnosis of "doubtful gonorrhea" was given. Gram-stained smears from these patients yielded a positive diagnosis in 13

³ München. med. Wchnschr., 1920, 67, p. 1067.

⁴ Collected Studies from Research Laboratory, Dept. of Health, City of New York, 1911, 6, p. 29.

⁵ Jour. Immunol., 1920, 5, p. 499.

instances, or in just one-half as many as did the cultural method. Of the 26 culturally positive cases, 5 gave positive films, 2 were suspicious, and 19 were reported as of the observation type. There were, accordingly, 8 cases yielding positive smears from whom the gonococcus was not isolated.

Twenty-six of the patients had received irrigations for longer or shorter periods before the cultures were made, although, of course, the local treatments were stopped 3 or 4 days before the specimens were taken. Of these treated patients only 3 yielded positive cultures, but 5 gave positive smears. Further, the 5 cases giving positive smears were not among those successfully cultured; of the latter, 2 gave the observation type of smear and 1 the suspicious type.

It should be noted that in some of these cases the plates were so overgrown with other types of bacteria that gonococci, even if present, would not have had an opportunity to develop. If a second examination had been made in some of these cases no doubt a somewhat higher percentage of positive results might have been obtained. On the other hand, the smears were not always of such a quality as to provide optimal conditions for examination.

These results are summarized in table 2.

TABLE 2
COMPARATIVE RESULTS WITH CULTURAL AND SMEAR METHODS OF EXAMINATION

Total Number of Cases	Cultural Results		Gram-Stained Smears			
	Positive	Negative	Positive	Suspicious	Observation Cases	Negative
92	26	66	13	17	56	6

From our results with these two methods of diagnosis in suspected gonorrheal infections of this type we may conclude that the cultural methods employed are likely to give a higher percentage of definitely positive findings than are the smear examinations, but on the other hand, the latter may be positive when cultures are negative. Of the 21 cases clinically diagnosed as "doubtful gonorrhea," 5 gave positive cultures and 3 positive smears and 4 suspicious smears, but 4 out of the 5 culturally positive cases yielded the observation type or negative smears. These two methods, thus, tend to supplement each other and are both of value in the control of these cases.

2. *Comparison of the Complement-Fixation Test with Smear and Culture in Relation to Clinical Diagnosis.*—In this section are reported the results obtained with complement-fixation tests by Miss Wilson of the Serologic Laboratory, Department of Health, the smear examinations at the Kingston Avenue Hospital, and the cultures by Mr. Buckell, on 56 of the 102 cases described in part 1.

The method for the complement-fixation test has been described.⁵ The essential points are the preliminary tests of individual guinea-pig serums for gonococcus fixability and careful titration of the selected, pooled complement with constant doses of antishoop amboceptor and 5% suspension of sheep cells.

TABLE 3
COMPARISON OF SMEAR, CULTURE AND COMPLEMENT FIXATION IN VARIOUS STAGES OF GONORRHEA IN WOMEN

Diagnosis	Number of Cases	Percentage of Positive Diagnoses		
		Smears	Cultures	Complement Fixation
Acute gonorrhea.....	1	Observation	100% positive	No complete fixation
Subacute gonorrhea.....	8	50%	50%	50%
Chronic gonorrhea.....	33	12%	20%	69.5%
Doubtful gonorrhea.....	14	14%	28%	71%

A summary of the tests of the 56 cases without reference to the clinical data, shows 16 cases giving positive cultures, or 27%, 10 giving positive smears, or 16.5%, and 37 giving fixation, or 66%. The fixations were divided into: 8 strongly positive, or 14%; 15 moderately positive, or 26.5%, and 14 weakly positive, or 25%. If we should consider the weakly positive reactions insignificant, we would still have 40.5% positive fixations in the 56 cases. In the discussion of the following tables we shall give our reasons for considering the weakly positive reactions to be diagnostic of present or recent gonorrheal infection.

The important point is to determine the relation of the laboratory tests to the clinical classification, and we give a comparison in table 3. The clinical classification is that used at the Kingston Avenue Hospital as described by Smith and Wilson.⁵ This classification cannot be taken as a hard and fast guide to the course of the disease because of the inability to obtain correct histories and dates of infection of the patients.

As was demonstrated by Schwartz and McNeil,⁶ in the acute and subacute stages of gonorrhea one would not expect to obtain as high a percentage of positive reactions as in the chronic stage.

It is of interest to compare our results with those of Smith and Wilson, who carried out a similar comparative investigation, using for the cultural work a glycerol-veal-horse-serum agar streaked with blood. Of a total of 50 cases, 7 were positive culturally, 14% ; 3 were positive by smear and 26 had smears of the observation type; 41 gave positive fixation, or 82%, with 8, or 16%, showing a strong positive reaction.

Wilson has made a further study (to be published) of 181 cases previous to the present series. The 181 cases were classified as:

54 subacute cases ⁷ having	46.5% positive smears, 49.5% positive cultures, 51.5% positive fixation.
85 chronic cases having	13% positive smears, 15% positive cultures, 76% positive fixation.
7 doubtful clinically	no positive smears, no positive cultures, 42% positive fixation.

Controls: 346 nongonorrheal patients gave no trace of fixation, and 4 normal laboratory workers gave no trace of fixation.

In these three studies the complement fixation reactions in chronic cases dropped from 82% in first study to 76% in second study and to 69.5% in the present study.

We have not been able to determine the exact reason for this decrease in the positive reactions, but the chief cause may have been due to the time in the course of the disease that the tests were made. The character of the patients in our service has changed somewhat during the past two years. At the time of our first study all prostitutes having venereal disease were sent to Kingston Avenue Hospital, while, for some time past, the first offenders and young girls, only, are sent there.

In table 4 is given a report of the laboratory tests on 16 of our culturally positive cases. In these the complement-fixation tests were made at the serologic laboratory. The remainder of our culturally positive cases had the complement-fixation tests performed at another laboratory and are not included in this table.

⁶ Am. Jour. Med. Sc., 1912, 144, p. 815.

⁷ Included in the 54 subacute cases are 11 diagnosed as acute on admission, but as these were not early acute they have been placed with the subacute cases.

TABLE 4
RESULTS OF COMPLEMENT FIXATION AND SMEAR EXAMINATIONS IN 16 CASES
CULTURALLY POSITIVE

Case	Complement Fixation			Smears	
	Positive	Negative	Doubtful	Urethra	Cervix
L. S.	+	Suspicious	Suspicious
L. Sen.	+	+	+
P. A.	3+	Observation	Observation
E. B.	—	..	Observation	Observation
N. C.	3+	Observation	Observation
M. D.	+	Suspicious	Suspicious
L. R.	—	—	..	Observation	—
L. D.	3+	Observation	Observation
A. R.	—	..	Observation	Observation
A. M.	+	Observation	Observation
A. Mac.	+	Observation	Observation
J. F.	±	Observation	Observation
J. V.	2+	Observation
V. M.	3+	—	Observation
R. Ca.	—	..	Observation	Observation
M. H.	2+	Observation	Observation

In table 5 is given a comparison of the laboratory tests in 43 culturally negative cases.

TABLE 5
RESULTS OF COMPLEMENT FIXATION AND SMEAR EXAMINATIONS IN 41 CULTURALLY
NEGATIVE CASES

Cases	Complement Fixation*			Smears†		Smear at Time of Culture	Culture Material
	Positive	Negative	Doubtful	Urethra	Cervix		
M. K.	—	..	Observation	Observation	Observation	Cervix
H. B.	+	—	—	Cervix
M. Kar.	±	+	—	—	Cervix
M. W.	—	..	Suspicious	Observation	—	Cervix
G. B.	2+	Suspicious	Observation	Observation	Cervix
A. H.	2+	Suspicious	Observation	Observation	Cervix
B. M.	2+	+	—	—	Cervix
E. W.	+	Observation	—	—	Cervix
M. A.	—	..	+	—	—	Urethra
M. S.	4+	Observation	—	Observation	Cervix
E. G.	2+	Observation	—	—	Cervix
T. F.	2+	Observation	—	—	Cervix
J. O'C.	—	..	Observation	—	—	Cervix
V. H.	3+	Observation	—	Observation	Cervix
H. Ba.	2+	Suspicious	—	Observation	Urethra
W. B.	—	..	Observation	—	—	Cervix
A. B.	—	..	Suspicious	Observation	—	Cervix
M. B.	+	Observation	—	—	Cervix
C. C.	3+	Observation	Observation	Observation	Cervix
M. C.	+	Observation	Observation	Observation	Cervix
M. D.	2+	+	Observation	—	Cervix
B. E.	2+	Observation	—	—	Cervix
M. G.	—	..	Observation	—	—	Cervix
H. A.	—	..	Suspicious	Suspicious	—	Cervix
V. J.	+	+	Suspicious	Suspicious	Gland
I. L.	3+	Observation	Suspicious	Suspicious	Cervix
D. M.	+	Suspicious	Suspicious	—	Cervix
H. P.	+	Observation	Observation	—	Urethra
P. F.	—	..	Observation	Observation	—?	Cervix
I. F.	2+	+	+	Observation	Cervix
M. A.	2+	Observation	Observation	—	Cervix
A. E.	±	Observation	Observation	—	Gland
V. R.	+	+	Observation	—	?
J. R.	—	..	—	—	—	Cervix
E. C.	2+	Observation	Observation	Observation	Cervix
H. M.	2+	Suspicious	Suspicious	?	?
M. M.	—	..	Observation	Observation	Observation	Cervix
E. W.	+	Observation	Observation	—	Cervix
E. LaR.	2+	Observation	Observation	—	Urethra
S. G.	—	..	Observation	—	Observation	Cervix
B. F.	2+	—	—	—	Urethra

* Result of fixation at height of reaction is given. From 3 to 23 tests were made on each case, with an average number of about 8.

† From 3 to 18 smears from each of these cases were examined with an average number of about 9. The result most suggestive of gonococcus infection is given.

DISCUSSION

The results of this investigation tend to confirm the general impression that neither clinical observations alone, cultural tests, gram-stained smears nor fixation tests, as single methods of diagnosis, can be relied on as guides to diagnoses of actual infection with the gonococcus in cases of suspected chronic gonorrhea in women.

Considering first clinical observations, we find that 9 of our 29 culturally positive cases harbored the gonococcus in the cervix uteri without exhibition on the part of the patients of symptoms definite enough to warrant a clinical diagnosis of gonorrhea. Also 7 other cases, diagnosed as doubtful gonorrhea, which were culturally negative, gave definitely positive complement fixation and two of them also positive smears. Conversely, among 33 cases given a diagnosis of chronic gonorrhea, there were 5 cases in which the clinical diagnosis was supported neither by the cultural test nor by repeated fixations and smears.

Diagnosis by cultural tests would be, of course, the preferred laboratory procedure if it might be carried out with the facility and confidence which that method enjoys as applied, for instance, to the diagnosis of diphtheria. This, however, is at present quite far from being the case. Although our cultural procedures, as applied to the diagnosis of chronic gonorrhea in women, have yielded results which seem perhaps to be better than any reported heretofore, among 41 culturally negative cases (table 5), which were carefully studied, there were 8 cases giving positive smears and 13 other patients who gave a 2+ to 4+ complement fixation. In justice to the cultural method, however, it should be noted that in 6 of these 8 smear-positive cases, the smears from the urethra alone were positive, whereas the material for culture was obtained from the cervix uteri; also that the results given in table 5 for fixation and smear examinations were the optimal ones of repeated tests, whereas in the great majority of these patients only a single cultural test was made.

As regards diagnosis by means of gram-stained smear examinations our results would seem to indicate that this method would be likely to yield a considerably smaller percentage of definitely positive diagnoses than the cultural methods which we have employed. The relation of the findings with these two methods is summarized on page 151. It is stated there that 19 of the 26 culturally positive cases gave only the observation type of smear and, on the other hand, that many of the culturally negative cases also gave this type of smear. These observation smears,

however, give no more definite information than their name implies, namely, merely denoting the presence of an inflammatory condition in the urethra or cervix uteri, due to some pyogenic organism, quite possibly to the gonococcus. It is not unlikely, in fact, that secondary infections of these localities by streptococci of low grade virulence or other similar organisms persisting after the disappearance of the gonococcus might well be accountable for some of these observation types of smears and hence, if much dependence is placed on this finding, the release of such cases might be delayed longer than is necessary.

As regards the fixation test, there has been included in our list of positive gonococcus diagnoses all of the one-plus or weakly positive reactions. This has been done because in Miss Wilson's two previous studies ^{5,7} it has been demonstrated that this test is highly specific and that even weakly positive reactions may be considered as diagnostic of a gonorrheal infection. In these studies it is reported:

1. No degree of complement fixation was obtained in cases known as nongonorrheal. Those controls included 345 cases diagnosed as nongonorrheal and 4 normal laboratory workers.

2. The reaction reported as one-plus represents a definitely positive result and has had, in some instances, a comparative reading by a representative of another laboratory of 2-plus or even higher.

3. Some of our one-plus reactions have persisted for from 5 to 11 weeks. These tests were carefully controlled and could not be considered as "doubtful" from the point of view of technic.

4. The clinicians of the Venereal Disease Service of the New York City Health Department, as the result of a long series of observations, have come to regard a one-plus reaction as diagnostic of gonococcus infection. To quote Dr. Barringer's opinion in a recent paper:⁸ "Further study of groups of cases with special reference to undetected foci of gonococcus infection may establish the fact that the complement fixation test is the surest means of estimating when a cure has been effected and that we are justified in keeping the patient under treatment as long as these tests remain positive."

It would seem, then, that in the chronic stage of gonorrheal infection and also in the clinically doubtful cases complement-fixation tests, carefully controlled, will give a much higher percentage of positive diagnoses than cultures or smears (table 3) and that this test constitutes at present the simplest and most effective single guide for the control of

⁸ N. Y. State Med. Jour., 1922, 22, p. 145.

such cases. On the other hand, it should be noted that some positive cases will be missed if dependence is placed on complement fixation and smear examinations alone. As is shown in table 4, four of the 16 culturally positive cases (25%) gave persistently negative fixation tests and also indefinite smears (observation cases). The clinical diagnosis for 3 of these cases was chronic gonorrhea and for the other "doubtful gonorrhea." Then, too, a persistent one-plus reaction may mean in some cases past rather than present infection with the gonococcus. We do not know how long the antibodies may linger in the body after the period of actual infection has terminated.

SUMMARY

By way of a general conclusion it may be stated that the smear, cultural and complement-fixation methods of diagnosis in chronic gonorrhea in women have all proved useful, and that their relative values correspond to the order in which they are named, the last being the most valuable. Whenever possible, however, each test should be carried out, as it is shown that they tend to supplement each other.

It would seem likely that the cultural methods utilized in this study might find their most useful application, as far as public health work is concerned, in controlling the period of detention of infected women undergoing treatment and also in determining when cases of vulvovaginitis in children may be pronounced cured. With all such patients, of course, local treatments should be stopped at least 4 or 5 days before the cultures are made. In women patients material should be obtained for culture from both the urethra and the cervix uteri. At best it must be admitted that the conditions essential for the isolation of the gonococcus from these chronic infections of women are exacting and can be met only by one experienced in bacteriologic technic and with the facilities of a well-equipped laboratory. In spite of the greatest precautions, too, the plates at times may become overgrown with contaminating bacteria. The method is also more time-consuming than are the smear and complement-fixation procedures. A positive report cannot be made, at the earliest, before 2 days and for a negative report from 4 to 5 days may be required. On the other hand, the isolation of the gonococcus from one of these patients answers the question of infection in an entirely definite way and, under certain conditions, the results obtained may well repay the time and trouble necessary for the application of these cultural procedures.